Experiment #1 protocol

- Prepare a starter by growing the bacteria DH5Alphaz1 with the plasmid pSB1K3-GB1-GB2 in LB medium + appropriate antibiotics at 37°C overnight (in a 50ml falcon).
- Transfer 1ml starter to 100ml LB containing Kan antibiotics (in 500ml Erlenmeyer flask). Make overall 2 Erlenmeyer flasks as described.
- Grow at 37°C until $O.D._{600}$ of 0.6 (Check the O.D after 90 minutes).
  - For the spectrofotometer O.D. test use 1ml of LB medium as blank and then measure the O.D. by using 1ml from the sample.
- Add IPTG 1M to one of the Erlenmeyer flask to get IPTG concentration of 1mM.
- Culture the bacteria for 4 hours. Usually the growth takes 3 hours but here there is an expression of few proteins in serial; first the T4 RNA Ligase and then the CM Resistance, so 3 hours as usual + 1 hour for recovery.
- Spread the bacteria on plate containing CM+Kan/CM+Kan+IPTG (depends on the culture)- 100ul+rest. 4 plates overall.
- Transfers 1ml culture to 100ml LB, do it for both cultures. Both LB with Kan+CM and only one with IPTG.
- Incubate the plates and the LB O/N.
- After O/N incubation:
  - Count colonies on plates.
  - Read O.D of the LB culture.

Expect higher O.D. and more colonies while inducing with IPTG.

Experiment #2 protocol:

- Make an O/N starter of the 3 following bacteria: colonies 8&9 and one with blank pSB1K3.
- Grow the TOP10 Bacteria (colonies 8&9 and one with blank pSB1K3) in LB+Kan+IPTG to 0.6-0.8 O.D.

*TOP10 bacteria are preferred on DH5Alphaz1 because of the higher probability for production of the CM Resistance protein due to less lacI expression, therefore the promoter will be open almost constitutively.

- Grow TOP10 bacteria with only pSB1K3 (no insert) on LB+Kan+IPTG.
- Transfer from the 3 cultures 1ml to 50ml LB+Kan+IPTG+CM.
  *Use CM concentrations of 10^{-4}, 10^{-5}, 10^{-6} (usually we do 10^{-3}).
- Grow O/N.
- Measure the O.D. of the cultures.
- Compare between the O.D. of colonies 8&9 to the O.D. of the bacteria with blank pSB1K3 (A higher measurement is expected if the experiment workes).